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Cellular Origin of Cancer: Dedifferentiation or Stem Cell Maturation Arrest?

by Stewart Sell[1]

Given the fundamental principle that cancer must arise from a cell that has the potential to divide, two major nonexclusive hypotheses of the cellular origin of cancer are that malignancy arises *a*) from stem cells due to maturation arrest or *b*) from dedifferentiation of mature cells that retain the ability to proliferate. The role of stem cells in carcinogenesis is clearly demonstrated in teratocarcinomas. The malignant stem cells of teratocarcinomas are derived from normal multipotent stem cells and have the potential to differentiate into normal benign mature tissue. A widely studied model supporting dedifferentiation has been the putative origin of hepatocarcinomas from "premalignant" foci and nodules induced in the rat liver by chemicals. However, the dedifferentiation concept for hepatocarcinogenesis is challenged by more recent interpretations indicating that hepatocellular carcinoma arises from maturation arrest caused by abherrant differentiation of determined stem cells. Either hypothesis is supported by the cellular changes that occur in the rodent liver after different hepatocarcinogenic regimens. The formation of foci and nodules from altered hepatocytes supports dedifferentiation; the proliferation of small oval cells with the potential to differentiate into either biliary ducts or hepatocytes supports arrested maturation of determined stem cells. It is now postulated that foci and nodular change reflect adaptive changes to the toxic effects of carcinogens and not "preneoplastic" stages to cancer. The stem cell model predicts that genotoxic chemicals induce mutations in the determined stem cell which may be expressed in its progeny. Proliferation of initiated cells is induced by promoting events which also allow additional mutations to occur.

Introduction

Although analysis of carcinogenic events by modern techniques of molecular oncology has greatly increased our understanding of how malignant transformation may occur, there are still basic questions concerning the cellular process from which cancer arises. Given the fundamental principle that cancer cannot arise in terminally differentiated cells but must arise from a cell that has the potential to divide, there are two possibilities: tumors arise from dedifferentiation of mature cells or from maturation arrest of immature stem cells.

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Embryonal Theory of Cancer

The "stem cell" or embryonal origin of cancer may have been the first generally accepted theory of the etiology of cancer (*1*). The embryonal rest theory of cancer has been attributed to Cohnheim (*2*). He proposed that cancers arose from displacement of embryonic cells. At some stage of embryonic life, cells become isolated or fixed while they still possess great energy. These cells normally become differentiated in the adult, but because of their isolation they manifest embryonic capacity for continued growth in the adult. Rippert (*3*) expanded the embryonic rest theory to include the possibility that cells expressing embryonic potential for growth could arise in the adult (dedifferentiation). However, Rotter (*4*) proposed that primitive sex cells might lodge anywhere outside the ultimate sex glands during development and serve as the origin of tumors. He also thought that the growth of epithelial tumors depended on primary changes in the underlying connective tissue, which allowed invasion and expansive growth of epithelium.

With the identification of cell-free filtrates (*5*) and exogenous chemicals (*6*) that could cause cancer, the idea that an infectious event caused dedifferentiation of mature cells to form cancers became more popular. The more recently developed concept of infection with oncogenic viruses or of activation of proto-oncogenes (*7,8*) as the causative events in cancer has been explained by action of oncogene products on adult cells causing transformation to malignant phenotype (dedifferentiation). This explanation is supported by the observation that transfection or activation of oncogenes can cause proliferative changes in apparently mature cells in tissue culture. However, the process of carcinogenesis *in vivo* includes proliferation of less differentiated cells, and cell lines in culture always contain a certain fraction of proliferating cells that may represent determined stem cells.

Stem Cell Tumors

The similarity of histologic appearance and growth characteristics between embryonic tissues and cancer is a major observation that supports the concept of a stem cell origin of cancer. In particular, the characteristics of teratocarcinomas, which produce a variety of undifferentiated as well as fully differentiated cells, are more consistent with a stem cell origin than dedifferentiation (*9*). Teratocarcinomas contain structures resembling presomite embryos (embryoid bodies) intermixed with virtually every recognizable fetal and adult tissue (*10,11*). Pierce et al. (*12*) showed that teratocarcinomas (embryonal carcinomas) not only contained malignant presomatic cells, but also included benign somatic tissue. In addition, the ability of transplantable teratocarcinomas of mice to differentiate into mature benign cells provides irrefutable evidence for the stem cell origin of this form of cancer (*13*).

Transplantable teratocarcinomas were first produced by Stevens (*14*) by injecting the cells from the genital ridge of F_1 mice into the testicles of parental mice. The normal genital-ridge cells grew in the testes and gave rise to solid teratocarcinomas that have now been passed for over 200 generations in inbred parental mice (Fig. 1). When growing in the recipient mouse, proliferating, malignant, transplantable teratocarcinoma cells differentiate into benign cells located in well-differentiated keratin pearls within the tumor (*16*). Transplantation of the tumor-producing core cells of malignant teratocarcinomas into normal blastocysts may result in birth of normal differentiated adult cells (*17–19*). Labeled embryonal-carcinomal cells localize preferentially in the mural trophectoderm of the blastocyst, in the primitive endoderm, and rarely in the inner cell mass. The carcinoma cells differentiate into differentiated cells in accordance with their localization (*20*). Thus, at least for this form of cancer, the malignant potential exists in the stem cell and may be controlled by environmental factors present in differentiating tissue.

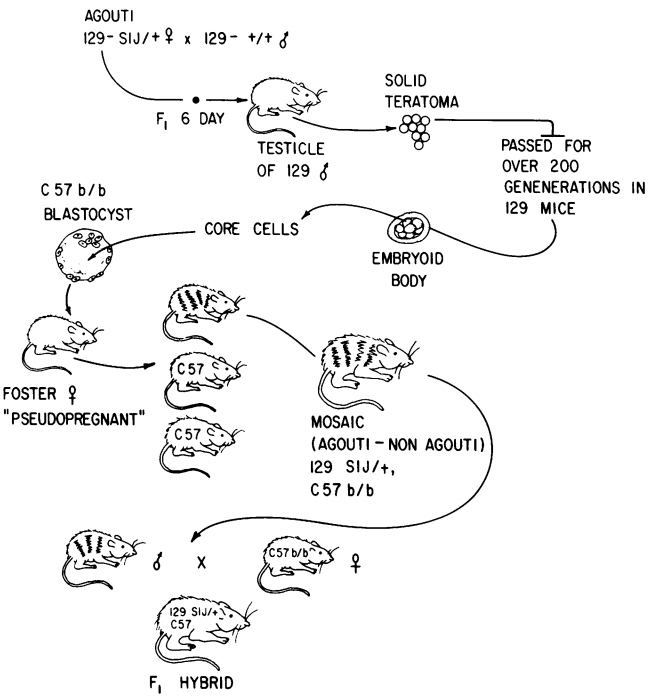

FIGURE 1. Origin of malignant teratocarcinomas from normal germinal cells and development of normal organs from transplantable teratocarcinoma cells. Malignant teratocarcinomas were derived by injection of normal genital-ridge cells of F_1 mice into the testicles of parental-strain mice. These teratocarcinomas have been maintained for over 200 generations by transplantation and may be grown in tissue culture. The malignant undifferentiated stem cells of the tumor (embryoid bodies) may be transformed into normal tissue after insertion into normal blastocysts of a different mouse strain and viable mosaic mice obtained. These mosaic mice have organs containing tumor-derived cells, as well as cells of the blastocyst, as determined by H_2 tissue type, red blood cell type, immunoglobulin allotypes, and isozymes from Sell (*15*).

Maturation Arrest/Blocked Ontogeny

The concept of blocked ontogeny, attributed to Potter (*21*), is a postulate of the stem cell model of carcinogenesis and cell renewal as adumbrated by Pierce et al. (*13*) In Figure 2, the undifferentiated stem cell is represented at the left. During organogenesis, stem cells differentiate to produce tissue stem cells. Stem cells that are committed to form a certain tissue are called "determined." The determined stem cells are the cells that are available to proliferate to form a given organ or cell lineage. The determined stem cells give rise to progeny that begin to accumulate the molecules of specialized cell types in their cytoplasm. During normal cell renewal the determined stem cell divides to produce two daughter cells. One daughter cell remains as a stem cell; the other daughter cell expresses a more differentiated state. These differentiating cells are capable of additional rounds of proliferation, eventually giving rise to terminally differentiated cells. Note that in Pierce's model the existence of a determined liver stem cell is highlighted by a question mark, and

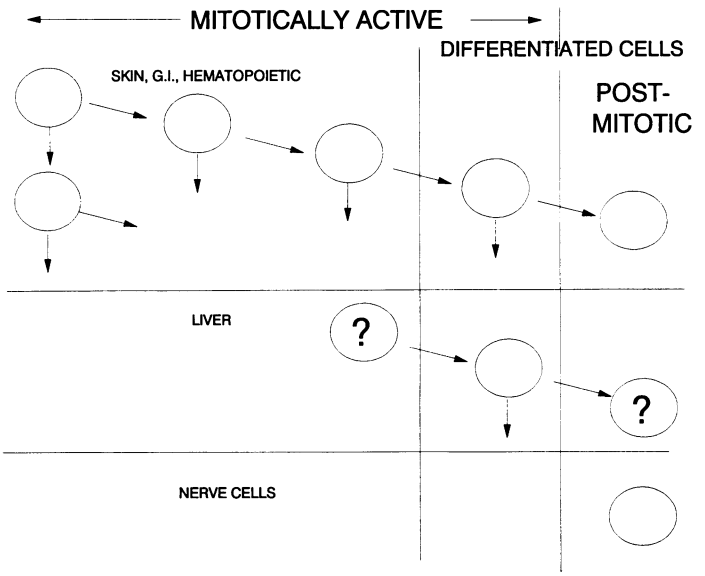

FIGURE 2. Model of cell renewal and carcinogenesis. The stem cell is depicted on the left with progressive differentiation to terminally differentiated cells at the right (post-mitotic). Expression of the malignant phenotype could occur at any stage of differentiation of mitotically active cells. Initiation may take place at the level of the stem cell, but tumors may appear to arise from more differentiated cells when additional mutations have occurred that permit expression of the malignant phenotype. The top lineage would hold for rapidly renewing populations of cells, such as the gastrointestinal tract, skin, and hematopoietic tissue. The second lineage represents tissues that normally turn over slowly, such as the liver. The question marks for the liver indicate that previously the ability of adult liver cells to proliferate and replace destroyed liver cells led investigators to conclude that there were neither stem cell nor terminally differentiated cells in the liver. However, more recent studies indicate not only that normal liver cell turnover involves periportal stem cells, but also that mature liver cells in the central zone are terminally differentiated. The bottom lineage depicts nerve cells of the adult, which are terminally differentiated, do not proliferate, and do not give rise to tumors. Modified from Pierce et al. (*13*).

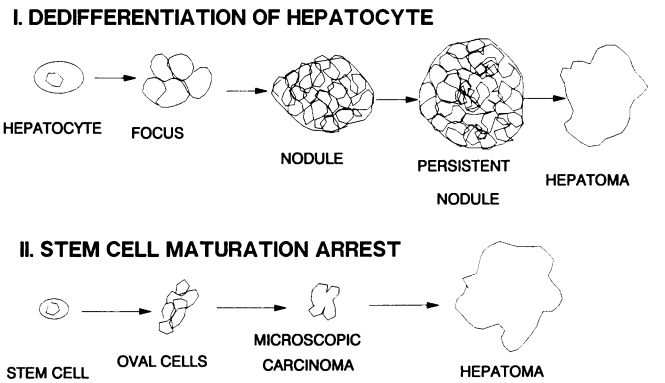

FIGURE 3. Possible cellular pathways to liver cancer. Two cellular lineages to liver cancer are presented: (I) dedifferentiation of mature hepatocytes and (II) aberrant differentiation of stem cells. The classic interpretation of the effect of chemical carcinogens in producing so-called premalignant foci and nodules implies dedifferentiation. The induction of proliferation of oval cells by chemical hepatocarcinogens is more consistent with derivation from stem cell maturation arrest (blocked ontogeny).

the presence of a terminally differentiated liver cell is also highlighted by a question mark. In this article the nature of the determined stem cell for the liver will be presented, as well as evidence that there are terminally differentiated liver cells.

Is There a Liver Stem Cell?

The question of whether there is a liver stem cell was posed in a recent review (*22*). The concept of the liver stem cell and its role in chemical hepatocarcinogenesis has developed from a number of studies of the cellular lineage of chemically induced hepatocellular cancer (*23–29*). There are two possible cellular lineages of cancer during chemical hepatocarcinogenesis (Fig. 3): tumors may arise by dedifferentiation of adult hepatocytes or by maturation arrest of stem cells. The sequence of foci to nodules to cancer and the associated changes in the enzyme content of nodular and carcinoma cells imply dedifferentiation of mature hepatocytes (*30–36*). However, an origin from stem cells is supported by the proliferation of small bile-ductlike cells that arise at the portal zone, proliferate extensively, and migrate between the hepatic cords to the central zone (*37–41*). These small bile-ductlike cells are called oval cells (*39, 41*; see Table 1). Oval cells contain markers such as α-fetoprotein (AFP). (*43–47, 53*) and epitopes identified by monoclonal antibodies (*53–56*), which are also found in hepatocellular carcinomas (HCC) that appear much later. Oval cells may differentiate into normal duct cells or hepatocytes (*41, 56, 58*) or migrate into persistent nodules where they may give rise to HCC (*55, 57*). The relationship of oval cells to the foci and nodules that develop during chemical hepatocarcinogensis was examined using four different models.

Carcinogen-Induced Oval Cell Proliferation

The effects of five carcinogenic regimens that show different kinetics of oval cell production, foci and nodule formation, and serum AFP elevations are compared in Table 2 and Figure 4. AFP is a serum protein found at high levels in fetal serum that becomes re-elevated in adults after liver proliferation or development of HCC (*20,59,60*). In our studies serum AFP levels were used to determine liver cell or oval cell proliferation early after carcinogen exposure, mitogen treatment (including partial hepatectomy), and necrotic liver injury, as well as development of liver cell cancer later after carcinogen treatment (Fig. 5). In recombinant, inbred mice, serum AFP may be used to predict which animals develop adenomas or HCC and to select animals for morphologic examination (*61,62*). Our first study used the cyclic AAF feeding regimen of Teebor and Becker (*63*). Although it was anticipated that AFP would be found in foci and nodules during the early ele-

Table 1.
Short history of oval cells and α-fetoprotein (AFP).

1944—Opie describes small cells in the liver after exposure of rats to butter yellow (*37*).

1954—Price et al. note similar cells after 3'methyl-4-dimethylaminoazobenzene exposure (*38*).

1956—Farber coins the term "oval cells" for the small cells (*39*)

1957—Popper concludes that oval cells arise from bile duct cells (*40*).

1964—Grisham and Porta conclude that oval cells may differentiate into bile duct cells or mesenchymal cells (*41*).

1964—Ruben, using autoradiography, concludes that oval cells can differentiate into hepatocytes (*42*).

1973-75—Onoe and coworkers localize AFP to oval cells early during hepatocarcinogensis (*43,44*).

1977-78—Tchipysheva et al. (*45*), Kuhlmann (*46*), and Sell (*47*), report AFP is not in foci or nodules.

1978—Shinozuka et al. report massive proliferation of oval cells in rats fed carcinogens in choline-deficient diets (*48,49*).

1980-82—Sell et al. propose oval cells as precursor cells for hepatocellular carcinomas and culture oval cells *in vitro* (*23,24,50*).

1984—Sell and Salman identify proliferating periportal cells early after carcinogen exposure with later ductal cell proliferation and suggest that this may be the stem cell for hepatocellular carcinoma (*51*).

1985—Yaswen et al. note increased expression of protooncogenes (c-*myc* and c-K-*ras*) in oval cells when compared to normal liver (*52*).

1989—Dunsford et al., using monoclonal antibodies, conclude that oval cells represent proliferation and differentiation of a liver stem cell and that foci and nodules are adaptive changes and not precursors to hepatocellular cancer (*55,56*).

1990—Sell proposes that liver stem cell may be either periportal cell or transition duct cell (*21*).

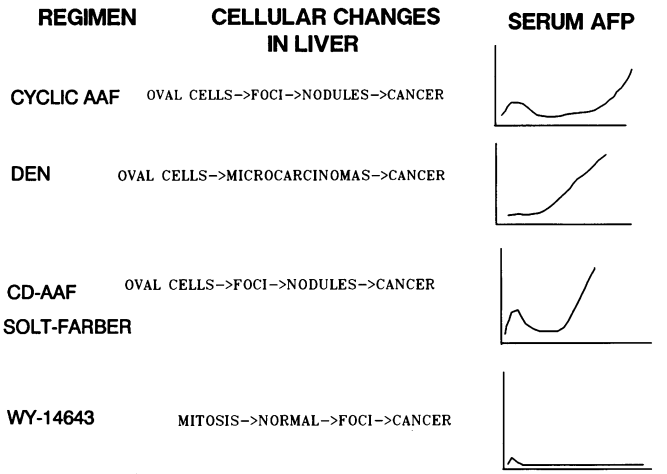

FIGURE 4. Cellular changes in the liver and kinetics of serum α-fetoprotein (AFP) elevations in rates exposed to different carcinogenic regimens (see text and Table 2). In the cyclic α-acetylaminofluorene (AAF), CD-AAF, and Solt-Farber models, early AFP elevation is associated with oval-cell proliferation and late AAF elevation with tumor development. The cellular changes and serum AFP elevation seen with the CD-AAF and Solt-Farber models are similar, except that the massive oval-cell proliferation and serum AFP elevation is delayed in the Solt-Farber model until AAF feeding stops. Dimethylnitrosamine produces little oval-cell proliferation early, but oval cells and transition cells between oval cells and atypical hepatocytes can be seen using monoclonal antibodies OV6 and T6 (*55*). WY-14643, a nongenotoxic peroxisome proliferator, induces liver cell proliferation (mitogenesis) and a small associated AFP elevation, but subsequent elevation of AFP has not been reported.

vation of serum AFP, it became clear that serum AFP became elevated before nodules appeared and that the cells containing AFP were oval cells and not foci or nodules (*43–47*). From these observations it was tentatively concluded that HCC might not arise from foci and nodules but from oval cells (Fig. 6).

A study of the early cellular events with dimethylnitrosamine (DEN) was most revealing regarding the cellular lineage of HCC (*56*). This model was chosen because there was little recognizable early oval-cell change and no early elevation of serum AFP, a marker associated with early oval-cell proliferation during carcinogen exposure (*60*). In this model, HCCs appear to arise from microscopic foci of atypical hyperplasia, rather than from either oval cells or preneoplastic nodules seen in other models. However, when monoclonal antibodies were applied to identify different cell populations, the early appearance of oval cells was readily detected (OV6+), and morphologic evidence of transformation of oval cells to larger cells bearing the HCC phenotype (T6) was seen (*56*).

Oval cells were also identified as the AFP–containing cells in the Solt-Farber model of inducing HCC. This

Table 2.
Oval cell proliferation and α-fetoprotein production in selected models of hepatocarcinogenesis in rats.

	Preneoplastic morphologic change		Serum AFP levels		
Carcinogen	Early	Late	Early	Late	Hepatomas
Cyclic AFF	Increasing oc, foci	Nodules, OC, ducts	++	+	+++/0
DEN	Little change	OC, microcarcinomas	0	++	++++
CD-AAF	Massive OC	Foci, nodules	+++	++	+++
DEN-AAF-PH[a]	Massive OC, foci	Nodules	+++	++	+++/+
WY-14643	Hepatocyte Mitosis	Microcarcinomas	+	0	0

Abbreviations: AFP, α-fetoprotein; AAF, *N*-2-fluorenylacetamide; OC, oval cells; DEN, diethylnitrosamine; CD, choline deficiency; PH, partial hepatectomy; WY, Wyeth.
[a]Solt-Farber model.

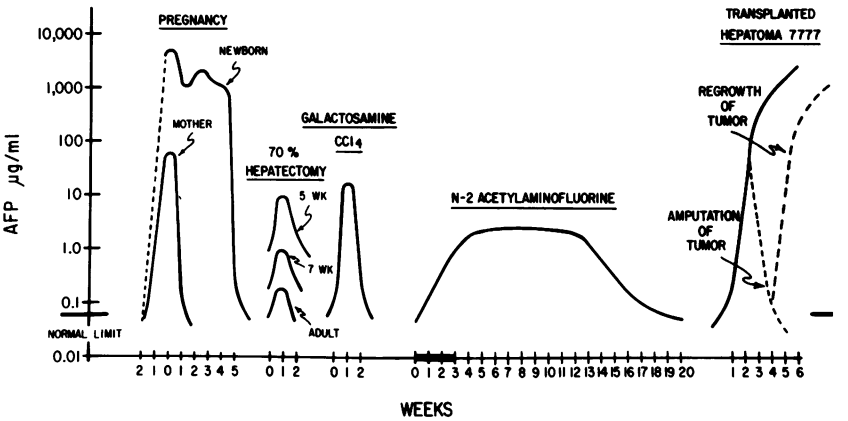

FIGURE 5. Serum α-fetoprotein (AFP) levels in rats. Elevated serum concentrations in mice and rats are found with events that induce normal or abnormal liver cell proliferation, such as development of the fetal liver, restitutive liver injury after partial hepatectomy or chemical injury, mitogen exposure (phenobarbital, peroxisome proliferators), exposure to chemical hepatocarcinogens, and growth of hepatocellular carcinomas. From Sell and Becker (*60*).

regimen was designed to produce rapid development of foci, nodules, and cancer (*65*). Cells are initiated by a nonnecrotic injection of DEN, followed by a 2-week feeding of AAF, with a partial hepatectomy performed after 1 week of AAF feeding. The AAF is given to inhibit proliferation of the noninitiated hepatocytes that would be stimulated by the partial hepatectomy (PH), thus allowing the growth stimulus of the PH to act on the DEN–initiated cells. However, this regimen not only induces foci and nodule formation, but also there is massive, early oval-cell proliferation. Later, cells with oval cell phenotypes may be seen within persisting nodules. This is interpreted to indicate that even when HCC arises within a nodule, it may have originated from the oval cell lineage (*56,58*).

The fourth regimen, feeding a choline-deficient diet containing AAF (CD-AAF), was chosen to identify the earliest proliferating cells after hepatocarcinogen exposure. This regimen induces HCC rapidly compared to other regimens and produces massive, early oval-cell proliferation associated with elevated serum AFP concentrations (*66*). Using autoradiography, it was found that the first cells to proliferate were located in the periportal zone, next to the bile ducts (*50*). After 3 days, labeling of bile duct cells was seen, and later many bile duct cells were labeled. The proliferating cells expanded across the hepatic acinus from the portal triad to the central vein within 1 month. Differentiation into both bile duct cells and small hepatocyte cells was apparent after 2-3 weeks. Thus, in these four different models of chemical hepatocarcinogenesis, selected because of different cellular changes preceding liver cancer, evidence for the development of cancers from oval cells in the liver was obtained (*27*).

WY-14643 stimulates peroxisome proliferation and is a nongenotoxic mitogen (*67,68*) that induces short-term proliferation of liver cells and elevations of serum AFP early after administration (*69*), followed by a prolonged period of little change in the liver. With prolonged administration, enzyme-altered foci appear (*70*) that are different from those induced by genotoxic chemicals that they do not contain increased carcino-

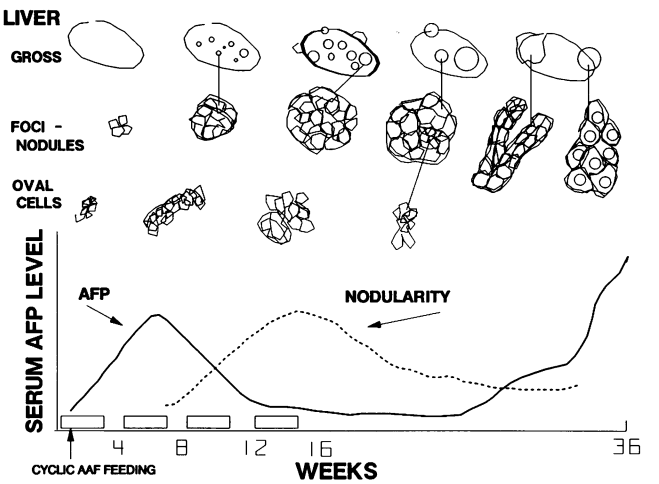

Figure 6. Morphologic changes and serum α-fetoprotein (AFP) levels during cyclic feeding of α-acetylaminofluorene (AAF). After four 2-weeks-on/1-week-off cycles of feeding AAF (a total of 12 weeks), rats develop one or sometimes two hepatocellular carcinomas 24 weeks later. After each AAF feeding the livers of the rats contain a progression of lesions from small foci of basophilic hepatocytes to larger eosinophilic foci to nodules of increasing size that distort the liver. If the fourth cycle is not fed, all of the changes are reversible, However, after the fourth cycle most of the nodules still disappear ("remold"), but hepatocellular carcinoma (HCC) develops 24 weeks later from what appear to be persistent nodules that do not regress (*64*). The progressive histologic and enzymatic changes in the foci and nodules preceding the appearance of HCC led a number of investigators to conclude that foci represent initiated hepatocytes that may be selected by further treatment to express the malignant phenotype (*32–36*). However, there is also early proliferation of oval cells, which differentiate into normal liver and bile duct cells as well as migrate into nodules and transform into hepatocellular carcinomas. Elevations of serum AFP are associated closely with oval-cell proliferation and tumor development, but not with nodule formation.

gen-detoxifying enzymes (*71,72*). Later, AFP-negative cancers are seen (*69*). Oval-cell proliferation has not been clearly defined using AFP or monoclonal antibody markers in this model, and the cellular precursors to cancer may be different from those of other regimens.

Where Is the Liver Stem Cell?

If there is a liver stem cell, where is this cell in the normal liver and what is its relationship to the oval cells seen during treatment with chemical carcinogens? Three candidates are the terminal duct cell (*40,41*) the transition duct cell (*22*), or a periportal stem cell (*51*). The relationships of these cell types is illustrated in Figure 7. Earlier autoradiographic studies implicated terminal bile duct cells as the precursors of oval cells (*40,41*). However, the conclusion that oval cells come from duct cells was based on autoradiographic studies done several weeks after carcinogen exposure (*41,74*). Using autoradiography during the first 3 days after CD-AAP, the majority of the proliferating-cell population was identified as a periportal cell, whereas after 3 days, labelling increased in the duct cell population (*50*). These early labeled cells were identified by electron microscopy as small periportal cells, and later duct cells, in particular, transition duct cells (*22,51*). Later, when oval-cell proliferation is at a peak, many of the oval cells infiltrating the liver cords are transition duct cells (*41*). It is possible that both the transition duct cell and a periportal-liver-determined stem cell have the potential to proliferate and differentiate into duct or liver cells. Which cell is predominant may depend on the degree of carcinogen stimulation or liver cell injury. The greater the stimulus, the more likely it is to involve the less differentiated periportal cell.

Bile Duct Proliferation

An argument against the terminal bile duct as the liver stem cell is that stimulation of bile duct proliferation per se does not result in hepatocarcinogenesis (*42*). Agents that selectively stimulate bile duct proliferation do not induce oval-cell proliferation. Induction of bile duct hyperplasia by nonhepatocarcinogens such as bile duct ligation, 4,4'-diaminodiphenylmethane (DDPM), or α-naphthylisothiocyanate (ANIT) do not induce AFP or albumin-containing duct cells (*75–77*). There is one report identifying AFP-containing bile duct cells after ANIT (*78*), but this was not found in our studies (*75,76*). Elmore and Sirica (*79*) have demonstrated that ductular cells may be stimulated to proliferate and differentiate into cells expressing mucin (intestinal metaplasia) or cells resembling hepatocytes

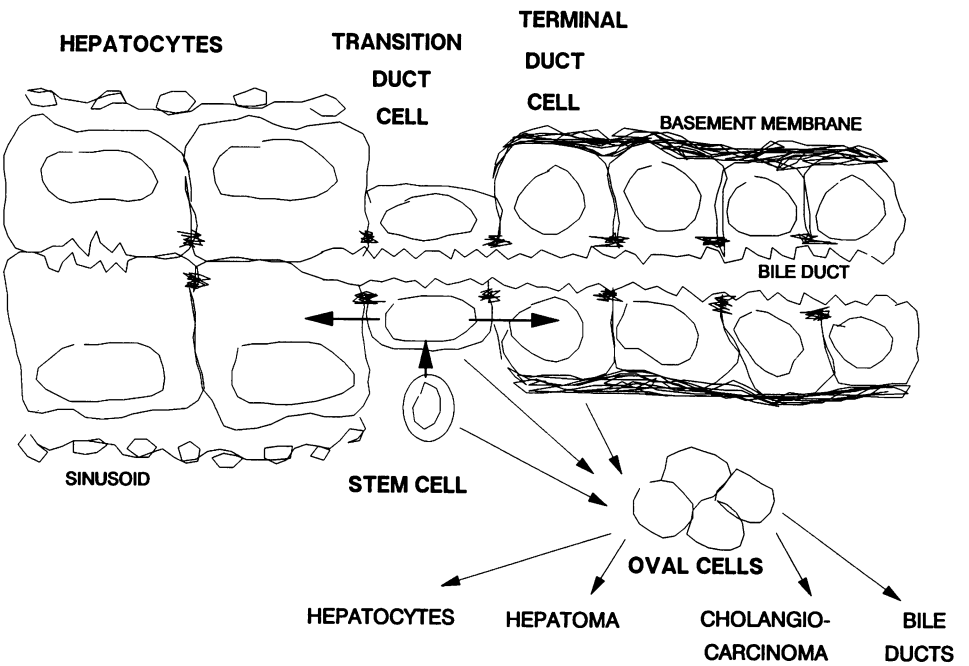

FIGURE 7. Normal and neoplastic stages of differentiation in the liver. This figure depicts the architecture of the junction between hepatocytes and bile ducts including the three cell types that have been implicated as giving rise to hepatocellular carcinomas: the terminal duct cells, transition duct cells, and the putative periportal liver stem cell. Transition duct cells have tight junctions with hepatocytes on one side and bile duct cells on the other (*73*). The thick arrows indicate the putative stages of restitutive cell renewal from periportal stem cells to transition duct cells to liver cells on one side and bile duct cells on the other. The thin arrows depict neoplastic proliferation. After exposure to hepatocarcinogens, many oval cells have extensive plasma membrane appositions between hepatocytes and bile-ductlike cells, including desmosome structures, tight junctions, and microvilli interdigitations (*74*). During cenogenesis, it is postulated that hepatocellular carcinomas arise from either the putative periportal stem cells or from the transition duct cells, or their progeny.

histologically, but not cells expressing hepatocyte proteins. This proliferation is most likely for bile-duct-determined stem cells at the stage of differentiation past that of the common stem cell for hepatocytes and duct cells, perhaps the terminal duct cell. On the other hand, the oval cells that appear after carcinogen exposure share bile duct and hepatocyte phenotypes (*50,75,76*). Thus, there appear to be two distinct types of ductlike cell proliferation (*80*): one is nonmalignant proliferation of bile ducts, the other proliferation of duct-like oval cells that have the potential to produce malignant tumors in the liver cell lineage.

Liver Stem Cell and Restitutive Proliferation

One of the properties of determined stem cells is to restore amputated or damaged tissue. For example, the stem cell for the limb of the amphibian is able to regenerate to form a normal limb after amputation, but it cannot form other organs (*81*). The presence of a liver stem cell has been questioned in the past because it did not appear that a liver stem cell was required for regeneration of the liver (Fig. 2). After PH or chemical injury (CCl_4, galactosamine) the liver deficit is replaced by proliferating hepatocytes that are derived from adult hepatocytes (*82–86*). For example, after two-third PH of the rat, each hepatocyte in the remaining lobes divides once or twice within a 48-hr period, without involvement of a stem cell (*83,84, 86,87*). In fact, AFP can be identified in large, dividing hepatocytes after PH in the rat (*88*) and adjacent to zones of liver injury after CCl_4 injury in the mouse (*59*).

However, with more severe liver injury, a liver stem cell may be called upon to restore the liver. For example, after severe injury with CCl_4, Engelhardt et al (*89*) found some small ovallike cells contained AFP, a marker for proliferation, and Petropoulos et al. (*90*) reported finding mRNA for AFP in small, nonparenchymal cells. Tournier et al. (*91*) found large amounts of AFP and mRNA for AFP after galactosamine injury in proliferating oval cells. Oval-like cells may be seen in human livers that have been removed after transplantation when the donor liver has suffered too much damage to restore normal function (J. Demetris, personal communication).

Stem Cells in Developing Liver

Determined stem cells are also the precursors for normal adult cells in developing organs (*13*). In the liver of the developing rat fetus, cells have been identified that have the characteristics of the oval cells seen in the adult (*53,58,88,92–94*). At days 12-14 of development, there are bipotential precursor epithelial cells that are capable of differentiation into hepatocytes or biliary epithelial cells (*95–97*). The ability to induce pancreatic duct cells to differentiate into liver cells (*98–100*) and for liver cells to differentiate into acini of cells containing zymogen granules (*101*) indicates that there is a common stem cell for liver, biliary ducts, and pancreas. Thus, there appears to be a determined stem cell in the fetus that gives rise to pancreas and liver.

Role of Stem Cells in Normal Liver Cell Turnover

There is some evidence that the cells that make up the hepatic cords are replaced by proliferating cells that originate in the portal area and migrate to the central zone, where they terminally differentiate and are removed by apoptosis (*102–104*). It is proposed that the heptocytes are formed at the periportal tract rim, where determined stem cells interact with ductal and stromal elements. The assembled unit then streams across the three acinar zones until it reaches the terminal hepatic vein where it is eliminated. The liver unit replaces itself in a manner similar to, but much slower than, the layered epithelium of the skin or bladder and the gastrointestinal tract (Fig. 8). In the skin, stem cells located in the basal layer of skin divide to produce daughter cells, one of which differentiates and migrates to the next layer and eventually terminally differentiates into a non-nucleated squamous cell (*28,105,106*). A steady number of gastrointestinal lining cells is maintained by a balance of cells proliferating in the lower levels of the epithelium to those differentiating in the mid-level of the epithelium and exfoliated at the surface (*107,108*). A crypt of the small intestine contains 4–16

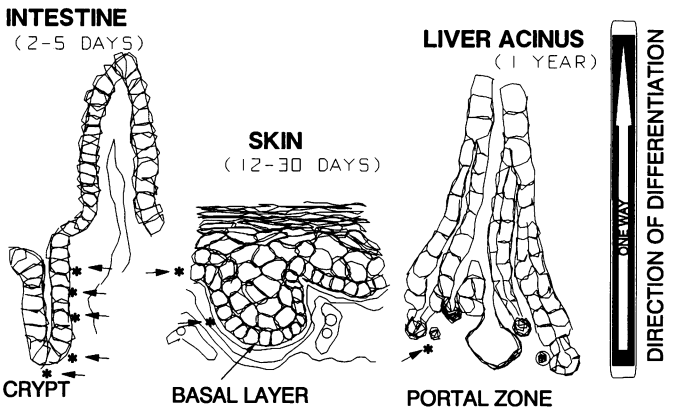

FIGURE 8. Comparison of cell renewal in intestine, skin, and liver proliferons. Normal cell turnover in the gastrointestinal tract, skin, and liver appears to proceed similarly, but at greatly different rates. The small arrows and asterisks denote the location of proliferating stem cells. Proliferating cells may be demonstrated in crypts of the gastrointestinal tract up to the opening of the crypt, limited to the basal layer of the skin, and rarely in the liver. Toxic or destructive events may increase the proliferation rate in these organs so that proliferating cells may be seen in higher layers in the skin and in the hepatic cords. Induction of proliferation of hepatic stem cells requires either massive loss of hepatocytes or inhibition of hepatocyte proliferation by a necrotic dose of genotoxic carcinogen.

actual stem cells in steady state, but up to 30–40 potential stem cells that may be activated to divide after perturbations that stimulate proliferation. In man, approximately 10^{11} epithelial cells are shed every day in the small intestine (*107*). It is estimated that the skin replaces itself every 15–16 days (Jean London, television commercial) whereas the liver may take over a year (*83*). The number of potential stem cells in the liver that may be activated has not been estimated; oval cells most likely represent progeny of activated stem cells. The characteristic of liver tumors may be related to the stage of differentiation at which the malignant phenotype is manifested (Fig. 9), as is postulated for teratocarcinomas and other cancers.

Chemical Hepatocarcinogenesis and Liver Stem Cells

Cohen and Ellwein (*108*) have recently emphasized the role of cell proliferation in the induction of cancer by chemicals. In considering the principle that two or more mutations must take place before the malignant phenotype is expressed (*109,110*), the role of chemicals in inducing HCC may be either to induce a mutation (genotoxic) or to stimulate proliferation (nongenotoxic), allowing internal or spontaneous mutations to take place. In this explanation initiators induce alterations in DNA and promoters stimulate proliferation of the initiated cells, increasing the likelihood for additional mutations to take place (*108*). Both promoters and "endogenous carcinogens" (*111*) stimulate cell proliferation and decrease the time that it takes to produce cancers. Endogenous carcinogens are essentially hormones that stimulate cell proliferation.

Lasting Effect of Initiation

One of the principles of initiation is that once a genotoxic event occurs, it will persist essentially for the lifetime of the animal (*112,113*). In skin carcinogenesis, it must be the stem cell that is mutated. Mutations that occur in cells that have begun the differentiation process will not develop into cancer because differentiated cells are committed to terminal differentiation and eventual death (*107,108*). This must be true for skin and gastrointestinal and other epithelial cancers, as the rapid turnover of these cells would remove call

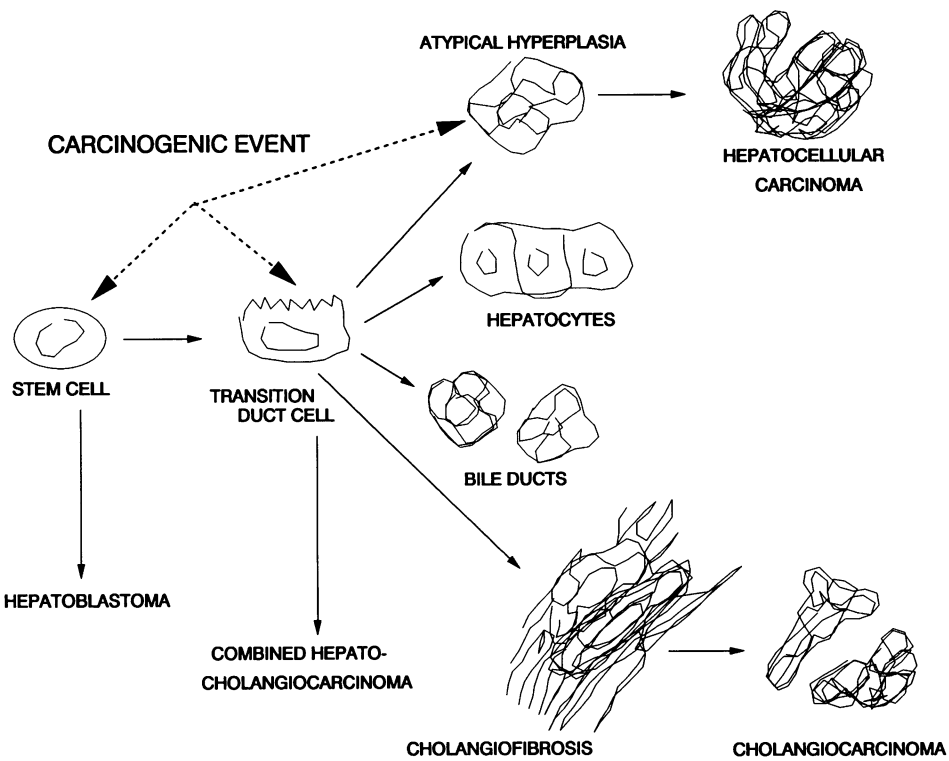

FIGURE 9. Postulated levels of expression of carcinogenic events during hepatocarcinogenesis. The stem cell model of hepatocarcinogenesis postulates that carcinogenic events occur in proliferating cells at some stage during differentiation, resulting in expression of the malignant phenotype (blocked ontogeny). Because carcinogenesis most likely results from the accumulation of more than one mutation, it is likely that the first mutation (initiation) takes place at the level of the stem cell and that later mutations occurring at the level of the transition duct cells or in aberrantly differentiating cells (atypical hyperplasia or cholangiofibrosis) direct the level of expression of malignancy. Hepatoblastoma may represent tumors that arise because of multiple mutations at the stem cell level. Tumors with combined features of hepatocytes and bile ducts (hepatocholangiocarcinomas) may arise from multiple mutations at a later stage of differentiation. Hepatocellular carcinomas arise from a still later stage of differentiation.

Table 3.
Effect of expression of hepatitus B virus (HBV) proteins in transgenic mice.

Transgene	Promoter	Effect	Reference
PSX	Albumin	Necrosis, hepatoma	(*124–126*)
PSX	Metallothionein	Necrosis, hepatoma	(*124,126*)
PSX	HBV PrS	No necrosis or foci, hepatoma	(*127*)
HBx	HBx transgene, enhancer	Foci, no necrosis, hepatoma	(*128*)
HBx	α-1-antitrypsin	Focal necrosis, no hepatoma	(*129*)

cells that have started the differentiation process within 2-5 days for gastrointestinal epithelium, 15-30 days for skin, and 50 days for pancreas (*114*). Although the cellular turnover in the liver is much slower (up to 1 year), it is likely that the same principle holds for liver cancer. It has been reported that the alterations induced by chemical hepatocarcinogens may also persist for long periods of time (*115–117*). For example, in unpublished experiments of Becker (personal communication), administration of phenobarbital as late as 1 year after injection of a non-necrotic dose of DEN (50 mg/kg), which by itself does not cause tumors, gave rise to primary HCC within 6 months. However, because of the long cellular turnover time in the liver, it is possible that a partially differentiated cell with the potential to proliferate might give rise to cancer after chemical initiation, thus fulfilling the maturation-arrest postulate of the differentiation theory of cancer (Fig. 2).

Hepatitis B, Hepatocellular Proliferation, and Hepatocellular Carcinoma

The incidence of cancer in different organs is closely related to the rate of cell turnover (*118*). The incidence of cancer of the skin and gastrointestinal tract is much higher than that of the liver in the western world. However, in areas of the world where hepatitis B is endemic and infected individuals have a marked increased turnover of liver cells related to liver damage, HCC is the most common cancer (*119,120*). The association of a higher incidence of HCC with hepatitis B infection is most likely related to the increased turnover of liver cells secondary to destruction of liver cells, leading to stimulation of proliferation of stem cells and other cells early in the liver acinus dedifferentiation proliferation (*121*). Similarly, increased restitutive proliferation in cirrhosis is also associated with increased incidence of HCC (*111,123*). This increased liver cell turnover increased the likelihood of endogenous carcinogenesis because of the increased chance for mutations (*108*). In transgenic mice expressing hepatitis B viral (HBV) proteins, liver cancer has developed both with and without preceding liver cell damage (*124–130*; Table 3). In addition, in transgenic mice that express hepatitis B surface antigen (HBsAg) and show progressive liver cell proliferation preceding cancer, exposure to chemical carcinogens such as DEN or aflatoxin results in HCC at a younger age (*131*). Thus, chemical hepatocarcinogens or insertion of HBV DNA at critical sites (*132–135*) may provide one mutational event necessary, but not sufficient, to cause malignant transformation. The increased proliferation of liver cells induced by cell injury or mitogens (promoters) allows accumulation of the other mutations required for expression of the malignant phenotype.

Relationship of Carcinogenic Stimulus to Proliferation of Stem Cells

Regardless of the cause of cancer, the carcinogenic process includes proliferation of stem cells or their determined progeny (*13,28,136*). Thus transforming viruses, genotoxic chemicals, nongenotoxic chemicals, tissue injury and repair, and developmental abnormalities that lead to cancer all induce proliferation of tissues requiring the participation of stem cells. In the case of teratocarcinomas, it is multipotent stem cell. In the case of cancer of different tissues, it is the tissue determined stem cell. It is possible that all cancers arise from some form of maturation arrest of stem cells. However, nature usually has more than one way to get to the same place. Both maturation arrest of determined stem cells and dedifferentiation of mature cells may be cellular pathways to cancer.

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